Primary Aldosteronism Takes (KCNJ)Five!

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Primary aldosteronism (PA) is the most common form of endocrine hypertension, due to autonomous aldosterone production from the adrenal cortex. Patients with PA typically present with hypertension, high plasma aldosterone levels associated with low plasma renin activity, and varying degrees of hypokalemia and metabolic alcalosis (1). The prevalence of PA increases with the severity of hypertension and is currently estimated around 10% in referred patients and 4% in primary care [(2) and references therein; (3)], but as high as 20% in patients with resistant hypertension (4-5). The two main causes of PA are aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH), also called idiopathic hyperaldosteronism. Efficient and timely screening for PA is of major importance, given the severe cardiovascular outcome of aldosterone excess that is independent of blood pressure levels (6-7). Yet, the pathogenic mechanisms leading to aldosterone hypersecretion and cell proliferation are largely unknown.

Recently, a few recurrent somatic mutations of the *KCNJ5* gene, coding for the potassium channel Kir3.4 have been implicated as a cause of APA, while an inherited mutation was identified in a family with a Mendelian form of early severe hypertension which features massive adrenal hyperplasia and is referred to as familial hyperaldosteronism type 3 [FH3, (8-9)]. These mutations all lie near or within the selectivity filter of the Kir3.4 channel; they result in a loss of channel selectivity, with increased sodium conductance leading to membrane depolarization. These changes are presumed to be responsible for constitutive aldosterone secretion and cell proliferation by promoting opening of membrane voltage-dependent calcium channels which is followed by activation of the calcium signaling pathway, the main trigger for aldosterone production in adrenal zona glomerulosa cells. In this issue of *Endocrinology*, Oki et al now formally establish a causal relationship between *KCNJ5* mutations and hyperaldosteronism. They demonstrate that the inherited *KCNJ5* T158A mutation produces a marked stimulation in aldosterone biosynthesis which is

dependent on membrane depolarization followed by calcium influx into adrenal cortical carcinoma cells (Oki et al, 2012).

The paper by Oki et al indeed tackles a central issue that had not previously been addressed, i.e. the causal link between *KCNJ5* mutations, membrane depolarization and aldosterone overproduction and cell proliferation. By transiently infecting adrenocortical HAC15 cells with a lentivirus expressing wild type KCNJ5 or mutated KCNJ5-T158A, the authors show that expression of channels harboring the T158A mutation potentiated basal aldosterone production, which was further stimulated by angiotensin II and the protein kinase A activator, forskolin. Using different fluorescent dyes, they confirmed the enhanced sodium influx through the mutated Kir3.4 channel, leading to membrane depolarization and increased intracellular calcium concentrations. KCNJ5-T158A transduced cells presented significantly increased expression of *CYP11B2*, the gene coding for aldosterone-synthase which ensures the last three enzymatic steps of aldosterone biosynthesis. Expression of *CYP11B1*, coding for 11β-hyroxylase, was also increased, as was basal and stimulated cortisol production. KCNJ5-T158A also induced significant production of 18-oxocortisol, a hybrid steroid largely produced in affected members of the original family with FH3 (10). *CYP11B2* expression and aldosterone production were both inhibited by calcium channel antagonists and calmodulin inhibitors, confirming the mechanistic link to activation of the calcium signaling pathway.

While these results confirm the pathogenic role of the *KCNJ5* T158A mutation in promoting aldosterone-overproduction, the same does not hold true for its effects on cell proliferation. Indeed, KCNJ5-T158A had an inhibitory effect (~30% reduction) on HAC15 cell proliferation, in stably infected cells, an effect which was not due to increased apoptosis and was unrelated to calcium signaling. Interestingly, a similar negative effect of *KCNJ5* mutations on cell proliferation was recently described for two other mutants. The *KCNJ5* G151R mutation is one of the recurrent somatic mutations found in APA and has recently

been described as inherited mutation in two families with early onset, severe progressive hyperaldosteronism and adrenal cortex hyperplasia, requiring bilateral adrenalectomy in childhood to control blood pressure (11). Despite this adrenal phenotype, *in vitro* studies demonstrated that the G151R mutation significantly reduced cell survival when transfected into human embryonic kidney 293T cells. A second mutation affecting the same amino acid, G151E, was recently described in a family diagnosed with non-glucocorticoid remediable familial hyperaldosteronism (12). This mutation was associated with a much milder phenotype of the two affected family members, compared to classical FH3, with blood pressure levels and hypokalemia easily corrected by medical therapy. Remarkably, the adrenals appeared normal by CT scanning, and hybrid steroids were produced at a rate comparable to that of other patients with sporadic PA. Again, the KCNJ5-G151E mutant channel was similarly permeable for sodium and potassium, resulting in depolarization of the plasma membrane and a continuous sodium influx (12). The KCNJ5 G151E mutation was reported in two additional families with early onset hyperaldosteronism of unknown cause (11). These subjects also had easily controlled hypertension and no evidence of adrenal hyperplasia. Remarkably, enough, in this study KCNJ5-G151E channels produced a much larger sodium conductance as compared to KCNJ5-G151R, resulting in rapid sodium-dependent cell lethality (11). Although G151E carriers do not present adrenal cortex hyperplasia, indicating that the mutation may limit adrenocortical cell proliferation yet promoting aldosterone overproduction, it is difficult to conceive how the G151R mutation, which also leads to reduced cell survival in the same study, induces bilateral adrenal hyperplasia when inherited and APA formation when occurring somatically. Based on these studies and the work by Oki et al, it remains therefore unclear whether and how *KCNJ5* mutations are responsible for increased cell proliferation. Alternatively, the observed effects raise the possibility that *KCNJ5* mutations are responsible for aldosterone hypersecretion,

while increased cell proliferation is triggered by other, yet to be identified, mechanisms. Consistent with this hypothesis is the observation that inactivation of several relevant adrenal potassium channels in mice leads to increased aldosterone production, but not to adrenal cortex hyperplasia or APA formation (Table 1).

Somatic *KCNJ5* mutations have been demonstrated to be present in a large proportion of patients with APA, with an estimated prevalence in unselected patients of ~34% (13), and even higher frequencies described depending on the sample size, the screening procedures for selecting patients for adrenalectomy and genetic background (14-15). However, although germinal *KCNJ5* mutations are responsible for inherited forms of familial hyperaldosteronism, they are not similarly causative for sporadic BAH (13). Even though unilateral adrenalectomy represents the treatment of choice for PA in specialized departments, the fact that only 30% of operated patients are cured and that a subset of patients don't even go into surgery because of the multi-tiered process for subtype identification, sets medical treatment as an attractive therapeutic option even in lateralized forms of PA. Kir3.4 potassium channels may thus represent interesting new drug targets for a subset of APA not eligible for surgery. Cell as well as animal models expressing mutant Kir3.4 channels may provide valuable tools to address the functional consequences of *KCNJ5* mutations, allowing to further dissect the mechanistic determinants of aldosterone overproduction and increased cell proliferation in PA.

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Table 1. Mouse models inactivated for potassium channels featuring adrenal aldosterone hypersecretion.

Inactivated channel (gene)	PA Phenotype	Adrenal cortex expression	Hyperplasia/ APA	Gender difference described	Ref
maxiK (*KCNMA1*/*KCNMB1*)	*KCNMA1* KO: Hyperaldosteronism, hypokalemia, normal renin levels; *KCNMB1* KO: hyperaldosteronism linked to renal K retention and hyperkalemia	*KCNMA1*: ZG>>ZF, ZR *KCNMB1*: adrenal gland	NO	NO	(16-17)
TASK1 (*KCNK3*)	Glucocorticoid remediable hyperaldosteronism, decreased plasma renin, hypokalemia,	ZG, ZF, ZR	NO	Females only	(18)
TASK1/TASK3 (*KCNK3*/*KCNK9*)	Hyperaldosteronism, reduced plasma renin,	TASK3: ZG	NO	NO	(19)
KvLQT1/IsK (*KCNQ1*/*KCNE1*)	Hyperaldosteronism in KCNE1 KO mice, normal renin, fecal sodium and potassium loss	ZG (*KCNQ1*, *KCNE1*)	NO	NO	(20)